PLANT CYTOLOGY

Apogamy in the Ferns.—It has long been known that the archegonia in a number of ferns are not functional and that in these forms the sporophyte generations arise as vegetative outgrowths from the gametophytes. This suppression of sexuality with the development of the succeeding generation asexually is termed apogamy. Only recently, however, have there been any cytological investigations of the phenomenon.

Farmer and Digby[1] were the first to study the nuclear behavior throughout critical phases in the life history of apogamous ferns. The results, based on forms of Lastrea, Athyrium, and Scolopendrium, led these authors to describe three conditions.

1. The process of sporogenesis is omitted from the life cycle in three varieties of *Athyrium Filix-fœmina* and in a form of Scolopendrium giving the condition of apospory known for a number of ferns. The prothallia arise directly from abortive sporangia or from pinnæ; the sporophytes develop apogamously from the prothallia or from unfertilized eggs; and the approximate number of chromosomes is retained throughout the life cycle. This type of life history brings apogamy into close association with apospory. The omission of the process of chromosome reduction, characteristic of sporogenesis, gives the gametophytes the sporophytic number of chromosomes (2x). Apogamy seems to be a natural consequence, for gametes would not be expected to function under such conditions since they would double the number of chromosomes with each nuclear fusion and there would be no reduction divisions to bring the higher numbers back to the normal. These conditions in the ferns agree with certain cases of apogamy among the seed plants (*Antennaria alpina, Thalictrum purpurascens*, and apogamous species of Alchemilla and Hieracium) where the reduction mitoses are omitted in the ovule and the nuclei of the embryo sacs contain the sporophyte number of chromosomes, the embryo developing from unfertilized eggs or even from synergids. The most interesting feature of this type of life history is the development of gametophytes with the 2x or sporophytic number of chromosomes, showing that the morphology of this phase in

[1] Farmer, J. B., and Digby, L. Studies in Apospory and Apogamy in Ferns. *Ann. of Bot.*, XXI, p. 161, 1907.

the life history does not depend upon its containing the reduced number.

2. In *Lastrea pseudo-mas* var. *cristata apospora* apogamy and apospory follow one another in the same manner as described above, but the number of chromosomes (probably 60) is so close to that of the gametophyte in the type species (72) that it seems probable that in this form the sporophyte retains the reduced number (x) of the gametophyte. This condition is exactly the reverse of that noted above showing that the morphology of the sporophyte likewise does not depend upon its containing the double number (2x) of chromosomes.

3. The third and most striking conditions described by Farmer and Digby refer to a peculiar migration and fusion of nuclei in the cells of the prothallium just before the apogamous development of the sporophytes. These observations are recorded from the study of two *polydactyla* varieties of *Lastrea pseudo-mas* which form their spores with chromosome reduction in the usual manner. The nuclear migrations and fusions occur in the younger regions of the prothallia, in the wings as well as in the thicker portions. A nucleus assumes an elongated form with the pointed end against the wall which it is about to pierce. A pore is formed through which the nucleus slips and makes its way to the nucleus of this receptive cell which usually remains rounded. The two nuclei come to lie closely pressed against one another and gradually fuse. Older prothallia thus have fusion nuclei with double the gametophytic number of chromosomes (2x) and the cells of the apogamously produced sporophytes are found to have nuclei of this type; the authors conclude that they are derived from such fusion nuclei. This process of migration and nuclear fusion, taking the place of the fusion of gametes, finds its analogy in the recent studies of Blackman and Christman on the rusts. Just previous to the development of the æcidia there is an extensive migration of nuclei between neighboring cells so that the cells which give rise to the chains of æcidiospores contain conjugate or paired nuclei, the descendants of which remain in pairs until the nuclear fusion in the teleutospores. Thus in the rusts and in these ferns a process of nuclear fusion concerned with vegetative cells has apparently become substituted for the fusion of gametes which are no longer functional.

The most recent cytological contribution to the study of apogamy in the ferns is by Yamanouchi.[2] This paper gives a much more detailed account of nuclear structure and the behavior of chromosomes than that of Farmer and Digby, and is remarkable for the thoroughness of the study of critical phases throughout the entire life history. We have already noticed a portion of the work in the review of some recent research on cilia-forming organs of plant cells in the August number of the NATURALIST. Yamanouchi worked upon *Nephrodium molle* which has the advantage of presenting under ordinary conditions of culture the normal life history of ferns. The apogamous development of sporophytes may, however, be readily induced in prothallia exposed to direct sunlight and watered from below so as to prevent the possible escape of sperms and fertilization of archegonia. Such prothallia develop much more slowly than under normal conditions. After six weeks the cushion regions become markedly thickened, which thickenings indicate the beginnings of apogamous sporophytes.

Yamanouchi made very accurate counts of the chromosomes throughout the critical phases of the normal life history preliminary to a comparison with apogamous conditions. The chromosome number in the sporophyte is 128 or 132, which is reduced during sporogenesis to 64 or 66 in the usual manner. The gametophyte has then 64 or 66 chromosomes which were counted in the vegetative cells of the prothallia and in the mitoses leading up to the formation of sperms and eggs. The fertilized egg has of course the double or sporophytic number.

Prothallia, which under the culture conditions described above produce sporophytes apogamously, have 64 or 66 chromosomes. The mitoses up to the 30–50 cell stages are similar to those in normal prothallia. After that the growth is very slow and there are irregularities in the position of the cell walls with reference to the surface of the prothallia. The apogamous prothallia produce antheridia in abundance which develop motile sperms, the mitoses showing 64 or 66 chromosomes. Archegonia are, however, rarely formed on apogamous prothallia. Occasionally archegonia initials are differentiated, from which a central cell is cut off as in normal prothallia, but this central cell either remains undivided or produces eggs and canal

[2] Yamanouchi, S. Apogamy in Nephrodium. *Bot. Gaz.*, XLV, p. 289, 1908.

cells in an archegonium with a poorly developed neck; it is doubtful whether such eggs are capable of being fertilized.

The sporophytic outgrowths on apogamous prothallia arise coincident with the development of the cushion region. Superficial cells on the underside increase in size, and from one of these an apical cell is cut off which becomes the growing point of a leaf. Meanwhile there is a rapid division of the neighboring cells in the interior so that an area of meristematic tissue results which gives rise to the young sporophyte in direct connection with the prothallial cells. A leaf and stem axis are developed from two superficial apical cells, the root tip arises endogenously, scalariform vessels appear in the tissue connecting the developing leaf and stem, and finally there is differentiated the young sporophyte with root, stem and leaf regions. Mitoses are easily found in stages of this apogamously developed sporophyte and always show 64 or 66 chromosomes, the gametophytic number of the prothallium. Consequently, in *Nephrodium molle,* there is no doubling of the number of chomosomes in the development of apogamous sporophytes through nuclear migration and fusion as described by Farmer and Digby for the *polydactyla* varieties of *Lastrea pseudo-mas.* It has not yet been determined whether these apogamous sporophytes develop spores.

Apogamy in Nephrodium, therefore, presents conditions different from anything as yet recorded for plants, since following normal sporogenesis a sporophyte is developed with the gametophytic or haploid number of chromosomes (x), and there is no place in the life history for the diploid or sporophytic number. The case of *Lastrea pseudo-mas* var. *cristata apospora* is apparently not the same since in that form apogamy follows apospory. However it is possible that the apogamous sporophytes of Nephrodium may be found at maturity to develop apospory and thus swing into a type of life history similar to that recorded by Farmer and Digby for the above form of Lastrea. The most significant results of Yamanouchi's investigation is the clear evidence that the morphology of the sporophyte does not demand that its cells contain nuclei with the double or diploid number of chromosomes (2x), in other words that the "number of chromosomes is not the only factor which determines the characters of the sporophyte and gametophyte," a conclusion indicated by the known cases in both ferns and seed plants

where gametophytes have the sporophytic number of chromosomes.

A third paper which should be mentioned in connection with these two on types of homosporous ferns is Strasburger's[3] study of apospory in heterosporous Marsilia. Parthenogenesis had been reported by Shaw as occurring in 50 per cent. of the female gametophytes of *Marsilia Drummondii.* Nathansohn had induced parthenogenesis in *Marsilia vestita* and *M. macra* by keeping germinating megaspores at a temperature of 35° C. for 24 hours and then allowing them to continue their development at a temperature of 27° C. Under this treatment the eggs of 7–12 per cent. of the spores gave rise to embryos parthenogenetically while at lower temperatures embryos were only developed after fertilization.

Strasburger found that in *Marsilia Drummondii* the nuclei of the female gametophyte contain 32 chromosomes which is the sporophytic or diploid number present in various vegetative regions of the sporophyte. The process of sporogenesis presents various irregularities: the number of megaspore mother-cells is less than 16 and at times only 4; sometimes the mitoses within these cells are reduction divisions of the usual type (heterotypic), but in other cases spores are formed only through vegetative mitoses in which the sporophytic or diploid number of chromosomes (32) is retained. Such spores give rise to female prothallia with eggs having the sporophytic number of chromosomes and a parthenogenetic development of the latter follows. These conditions differ from those of apospory in the fact that spores are developed, but agree in the final result that the process of chromosome reduction is suppressed in the life history. The microspores showed irregularities in their development and on germination did not produce mature sperms. Two other species in the genus, *Marsillia macra* and *M. Nardu*, presented similar conditions.

Perhaps the most important feature of this cytological research on apogamy is its bearing on current theories of the nature and basis of alternation of generations in plants. It is perhaps rather generally held by those who accept the antithetic theory that the differences between sporophyte and gametophyte are in some way concerned with the number of chromo-

[3] Strasburger, E. Apogamie bei Marsilia. Flora, XCVII, p. 123, 1907.

somes, the sporophyte taking its peculiarities because of the doubling of the number which results from the sexual fusion of gamete nuclei, and giving up these characteristics when the number of chromosomes are reduced at the end of the sporophytic phase. This view that nuclear structure and more particularly the number of chromosomes gives the physical basis for alternation of generations was originally stated by Strasburger and has received support from a large amount of research on life histories throughout the plant kingdom. It has in the opinion of some authors reached the stage worthy of statement as a law of development, as indicated by the expression x and 2x generations applied to gametophytes and sporophytes.

However, the cytological investigation of apogamy in the seed plants as well as in the ferns has shown for a considerable number and wide range of forms that the gametophyte generation may have the sporophytic number of chromosomes, and now in Nephrodium there is established the first instance in which a sporophytic generation may develop with the gametophytic number.

This evidence may be regarded by some as cutting at the roots of the antithetic theory of alternation of generations, but this does not follow. It is clear that an increase or decrease in the number of chromosomes within a certain range does not affect the morphology of the phase of the plant's life history concerned, and the cause of the specific characters of gametophyte and sporophyte must rest upon other factors. What these may be is problematical; it is not unlikely that a variety of factors is concerned. It is probable that the peculiarities of every species demand at least a certain amount of chromatin with a specific composition, but there is no reason to assume that this must be contained in a fixed number of chromosomes, and furthermore multiples of the minimum amount required would not be expected to introduce new characteristics except as it might give increased vigor or vitality. Then there is the cytoplasm to be considered and perhaps of even greater importance the complex reciprocal relations that must exist between the nucleus and cytoplasm.

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